Note on the Sex of a Tadpole raised by Artificial Parthenogenesis.

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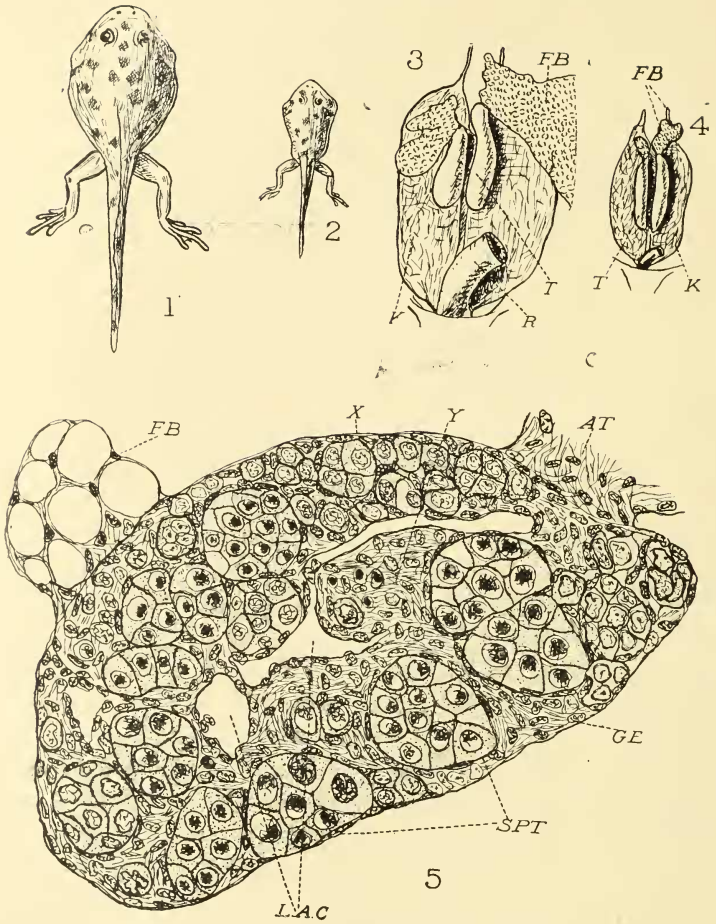
With 5 Text-figures.

With the object of ascertaining what is the sex of tadpoles of R. temporaria raised by artificial parthenogenesis I undertook some experiments last April. As my intention was to procure as many tadpoles as possible, I adhered to the method of smearing the eggs with a mixture of blood and lymph and then pricking each one with a very fine glass needle. The usual precautions were taken in this work, even the water in which the eggs were raised being drawn from a tank where it had remained for several days; the frogs were carefully washed in alcohol before opening, and the eggs were not allowed to touch the skin while being withdrawn from the swollen uterus.

Two sorts of glass needles were used; one was drawn from glass tubing and the other from solid glass rod; the former gave a higher percentage of burst and spoilt eggs, but while the latter sort of needle gave fewer irretrievably ruptured eggs, the percentage of successful segmentations was lower. There is little doubt that the minute lumen left in the glass-tube needle served to introduce more of the blood and lymph into the egg, and hence to promote segmentation. In some experiments carried out by the late Dr. Jenkinson different



TEXT-FIG. 1.—Parthenogenetic tadpole three months old. × 1.

TEXT-FIG. 2.—Control fertilized tadpole at same age raised under same conditions. × 1.

TEXT-FIG. 3.—Gonad and surrounding organs of the parthenogenetic tadpole. *F. B.* Fat body. *K.* Kidney. *R.* Rectum. *T.* Testis. × 10.

TEXT-FIG. 4.—Gonad and surrounding organs of control male. × 10.

TEXT-FIG. 5.—Obliquely longitudinal section of part of the gonad and fat body (*F. B.*) of the parthenogenetic tadpole. *A. T.* Attachment of gonad to roof of peritoneal cavity. *G. E.* Germinal epithelium. *L. A. C.* Lacunæ in gonad. *S. P. T.* Spermatic tubules. × 270.

fluids were injected into the egg, but though very large numbers were treated, only one abnormal tadpole was procured. The data got from these experiments and from those since carried out by myself seem to show that there are almost certainly other factors in the problem, as, for instance, in one batch of eggs pierced by a solid needle a very good percentage of tadpoles was got, while in another lot pierced by a hollow needle, not one even segmented. Nevertheless the whole series of experiments clearly showed in my case that the hollow needle was the better. Individual frogs differed markedly in the number of tadpoles raised from their eggs.

I pricked five thousand eggs of R. temporaria and raised about fifty tadpoles to the closure of the neural folds. There were, as is usual, many abnormal specimens, and the death-rate of those which hatched was high. Without going into details, it may be mentioned that fifteen tadpoles were raised to a stage when the external gills become covered by the epidermal overgrowth. Two of these were scarcely able to swim, and they soon died. Of the remainder all died except two, just before their hind limbs broke through. Those which died at this time did so, I believe, because the weather was most inclement, for the tadpoles born under natural conditions in the ponds were extremely backward for the season of the year. One of the survivors died at the critical period when the germ cells were beginning to become grouped in the manner which shows their sex. I believe this one would have been a male, but there was still undifferentiated material in the gonad. The sole survivor grew at a great pace and quickly outstripped the controls, so that it was nearly two and a half times normal size. In Text-fig. 1 and 2 are natural size drawings of this tadpole and a normal control raised n the same way; the parthenogenetic tadpole is normally proportioned, its hind limbs, tail, fæces, and its general outward morphology being proportionately large. The rectum, as was shown by the size of its fæces, and as subsequent dissection showed, was also very large.

At the age of three months the tadpole was placed in an

aquarium from which it was known normal tadpoles could not escape. To my regret I found that just as the front limbs had broken through, the tadpole jumped out on to the floor, where it died before I discovered its plight. In figs. 3 and 4 are drawn the gonads (*T*), kidneys (*K*), and rectum (*R*) of the parthenogenetic and normal tadpole respectively. When I sectioned the gonads of the former, I found that it was a well developed male, as the external appearance seemed to show, for the gonads were distinctly testiculiform.

In section the germ cells are clearly marked into numerous incipient spermatic tubules; though the section drawn in fig. 5 was across the least well-differentiated region, the spermatogonial nests are well marked. Undoubtedly the gonad had passed beyond the indifferent stage during which it is impossible to speak with certainty as to the sex. The part marked *X* in fig. 5 contains germ cells which have just begun to form spermatogonial groups, while that marked *Y* is apparently nothing more than a non-germinal core, the cells and their nuclei staining like the tissue forming the mesorchium (*A. T.*).

I feel quite sure that this tadpole was a male. Mr. Goodrich, whom I have to thank for his usual kind interest and suggestions, lately drew my attention to an abridged account of a paper by J. Loeb read before an American philosophical society on the same question as that dealt with in this note. Loeb found that the sex of an American species of parthenogenetic frog a year old was male. I have not yet seen Loeb's paper, but his results agree with mine as to the sex.